Spectral and Polarization Sensitivity of the Dipteran Visual System

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ABSTRACT Spectral and polarization sensitivity measurements were made at several levels (retina, first and third optic ganglion, cervical connective, behavior) of the dipteran visual nervous system. At all levels, it was possible to reveal contributions from the retinular cell subsystem cells 1 to 6 or the retinular cell subsystem cells 7 and 8 or both. Only retinular cells 1 to 6 were directly studied, and all possessed the same spectral sensitivity characterized by two approximately equal sensitivity peaks at 350 and 480 nm. All units of both the sustaining and on-off variety in the first optic ganglion exhibited the same spectral sensitivity as that of retinular cells 1 to 6. It was possible to demonstrate for motion detection and optomotor responses two different spectral sensitivities depending upon the spatial wavelength of the stimulus. For long spatial wavelengths, the spectral sensitivity agreed with retinular cells 1 to 6; however, the spectral sensitivity at short spatial wavelengths was characterized by a single peak at 465 nm reflecting contributions from the (7, 8) subsystem. Although the two subsystems exhibited different spectral sensitivities, the difference was small and no indication of color discrimination mechanisms was observed. Although all retinular cells 1 to 6 exhibited a preferred polarization plane, sustaining and on-off units did not. Likewise, motion detection and optomotor responses were insensitive to the polarization plane for long spatial wavelength stimuli; however, sensitivity to select polarization planes was observed for short spatial wavelengths.

INTRODUCTION

Previous spectral studies of the dipteran compound eye have centered on the spectral sensitivities of optomotor and phototaxic responses (1–3), electroretinograms (ERG's) (2), retinular cell potentials (4), discharge responses of interneurons in the third optic ganglion (5), and on spectral absorption properties of individual rhabdomeres (6, 7). Although some inconsistencies in the results have appeared, it is well established that dipterans are visually sensitive to near-ultraviolet radiation and insensitive to wavelengths longer than 650 nm. The microspectrophotometric studies (6, 7) indicate that of the eight retinular cells comprising one ommatidium, cells 1 to 6 possess the same

spectral absorption characteristics, which differ from those of cells 7 and 8. A retinal dichotomy is further supported by morphological differences (8) between retinular cells 1 to 6 and cells 7 and 8 which, coupled with the distinctly different projection pattern of their axons, suggests that the visual system is divided into two subsystems, one served by retinular cells 1 to 6 and the other by retinular cells 7 and 8.

Although it has not been demonstrated from behavioral experiments that flies can discriminate wavelength as do other insects (9, 10), recent studies (1, 11) of the optomotor response have provided support for the notion that the visual system can be divided into a (1–6) subsystem and a (7, 8) subsystem. Since the two subsystems have different spectral characteristics, a necessary condition for some form of wavelength discrimination capability is satisfied. The existence and degree of wavelength discrimination would depend upon the type of neural interaction occurring between the two subsystems. The levels in the visual nervous system where the two subsystems interact, the nature of the interaction, and the spectral sensitivity of the two subsystems are pertinent areas to be investigated.

Another interesting question concerns the exceptionally high spectral sensitivity in the ultraviolet (UV) region. It is not surprising that flies are sensitive to UV, for most insects exhibit a high sensitivity to such radiation; however, previous studies indicate that, unlike other insects, flies possess a sensitivity maximum in the UV region only in retinular cells that also have a sensitivity maximum in the visible region of the spectrum (4). Retinular cells having two spectral sensitivity maxima have also been reported in honeybees and dragonflies, but in these two insects, unlike the fly, retinular cells with a single sensitivity maximum in the UV region have also been observed. It therefore appears that the fly retinular cells contain either a photopigment having two visually active absorption peaks or an allotment of both a UV photopigment and a green photopigment.

Another distinctive characteristic of the arthropod compound eye is its ability to detect the plane of polarization. It is known that retinular cells of flies have a preferred plane of polarization, but just how this information is carried and processed by interneurons is still unknown. Since only two orthogonal polarization channels are necessary for the detection of the plane of polarization, the (7, 8) subsystem is suspected to carry such information, for the rhabdomeres of retinular cells 7 and 8 have their microvilli oriented orthogonally. It is interesting, therefore, to ask whether the (7, 8) subsystem does indeed carry polarization information and whether such information is lost in the (1–6) subsystem as is suggested by the neural anatomy of the first optic ganglion (12, 13).

Answers to such questions were pursued by studying the spectral and polarization sensitivity at various points in the visual nervous system under

conditions that would separately accentuate the influence of the (1–6) or the (7, 8) subsystem. Retinular cells were studied by measuring their spectral and polarization sensitivities as well as identifying the subsystem to which they belonged by intracellular staining techniques. Similar measurements were also made from the discharge behavior of two classes of units in the first optic ganglion, a class of motion detection units in the third optic ganglion, a similar class of units in the cervical connective, and from optomotor flight torques. Since successively higher levels of the nervous system reveal different and usually more complex properties of pattern recognition, it was considered important to employ monochromatic stimulus patterns which maximized the response at each level, such as monochromatic moving striped patterns for the motion detection units. At levels of the nervous system where the two subsystems interact, the spectral and polarization characteristics of each subsystem were studied by making use of the fact that rhabdomeres of retinular cells 7 and 8 have a smaller cross-section than do those of retinular cells 1 to 6 which implies that retinular cells 7 and 8 have a more restricted receptive field. By using moving striped patterns with a spatial wavelength of about 3°, the contribution from the normally dominant (1–6) subsystem could be sufficiently attenuated relative to that from the (7, 8) subsystem so as to make the influence of the (7, 8) subsystem dominate.

MATERIALS AND METHODS

Three species of flies were used, wild-type and white-eyed *Musca domestica*, wild-type and white-eyed *Calliphora erythrocephala*, and wild-type *Phaenicia sericata*. Each species provided an advantage for a particular type of experiment and was therefore predominantly used for that experiment. With greater difficulty it was also possible to perform each experiment with the other species. No species differences were observed with the exception that reverse optomotor reaction at short spatial wavelengths could only be elicited from *Musca domestica* and therefore the contribution from the (7, 8) subsystem could only be observed in this insect. All species were bred and raised in our laboratory, and experimental specimens ranged in age from 3 to 12 days post-emergence.

Retinular Cell Experiments

Intracellular recordings were made with glass micropipettes filled with either 2 M potassium citrate or 6% Procion yellow (M-4RAN, Colab Laboratories, Inc., Glenwood, Ill.) and having a resistance of 100–500 MΩ. Micropipette signals were amplified by a conventional high input impedance, capacity neutralization amplifier and were stored on magnetic tape for later reproduction and analysis. When dye was injected into the cell, the current passing through the micropipette was monitored as well. The micropipettes were lowered vertically, and penetration of retinular cells was facilitated by using a preparation jolting device similar to that described by Tomita et al. (14).

Specimens were chosen from white-eyed *Calliphora erythrocephala* because the penetrated retinular cell could be stimulated without accurate alignment of its optical axis with that of the stimulus generator as is necessary in wild-type *Calliphora erythrocephala*. Their heads were removed and carefully bisected horizontally with a razor. The ventral half was immediately placed in a high humidity chamber to reduce the drying that would rapidly occur in the open air. After injection of Procion yellow the head was left in the humidity chamber for 15–30 min to allow the dye to diffuse throughout the cell. The heads were fixed overnight in Bouin's solution (pH 4.0) and embedded in either paraffin or Maraglas (Polysciences, Inc., Rydal, Pa.). 15-μ sections were later observed and photographed through a Zeiss fluorescent microscope.

The stimuli consisted of pulses of monochromatic and polarized light and were generated by the apparatus diagrammed in Fig. 1 *a* Light emitted by a 120 w xenon arc lamp, S_1, (Bausch & Lomb Inc., Rochester, N. Y.) was condensed to the plane of the shutter (*Sh*). Much of the infrared component was removed by filter F_1. A photodiode placed behind the shutter provided a signal corresponding to the stimulus presentation which was recorded on magnetic tape. The stimulus intensity was controlled by a 4 log unit neutral density circular wedge, *W* (Eastman Kodak Co., Rochester, N. Y., Inconel [Huntington Alloy Products Div., The International Nickel Co., Inc., Huntington, W. Va.] on quartz), which was coupled to a precision potentiometer for providing an analogue voltage of the wedge position. The light was collimated into a beam 8 mm in diameter by the quartz achromat lens, L_1. The beam passed through a filter, F_2, consisting of one of 20 interference filters (Rolyn Optical Co., Arcadia, Calif.) and a 10% mirror, *M*, (Eastman Kodak Co., Inconel on quartz) before striking the fly's head. A rotary polarizer, *P*, could be inserted into the beam. A steady adapting light could be added to the primary beam through the second optical pathway which consisted of a 6 v incandescent source, S_2, collimating lens, L_2, and interference filter, F_3. Only visible wavelengths could be generated by the second pathway. The spectral calibration of the primary beam was made with a thermopile (Chas. M. Reeder & Co., Inc., Detroit, Mich., RBL-500) connected to a Keithley microammeter (150 B, Keithley Instruments Inc., Cleveland, Ohio).

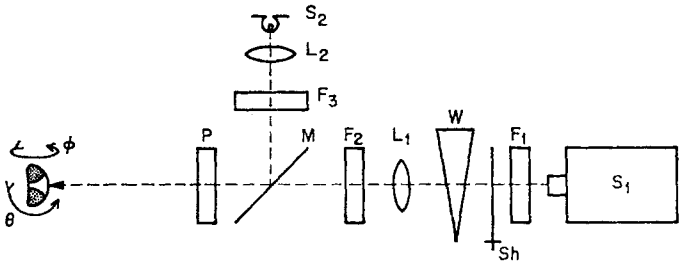

FIGURE 1 *a*. Schematic diagram of one of the two stimulus systems. System A (Fig. 1 *a*) was used in retinular cell and chiasma unit experiments and system B (Fig. 1 *b*) was used in motion detection and optomotor experiments. S_1, xenon source; S_2, incandescent source; F_1, heat filter; *Sh*, shutter; *W*, neutral density wedge; *N*, neutral density filter; F_2, interference filter; *P*, polarizer; *D*, quartz diffuser; $L_{1,2}$, quartz lens; *M*, 10% quartz mirror; F_3, interference filter.

Intermediate Chiasma Unit Experiments

Low impedance tungsten microelectrodes were used to record spike potentials from two distinctly different classes of units in the intermediate chiasma which joins the first and second optic ganglia. A description of these microelectrodes appears elsewhere (15). The potentials were amplified by conventional means. Wild-type *Phaenicia sericata* and *Calliphora erythrocephala* were used. Specimens were prepared by securing them intact to a ball-joint stand using dental wax applied with a hot Nichrome wire loop (Driver-Harris Co., Harrison, N. J.). The head was tilted forward and a triangular flap of exoskeleton was removed from the right posterior surface of the head capsule, thereby exposing the first, second, and third optic ganglia. The stand and preparation were then attached to a special platform carrying the micromanipulator and tungsten microelectrode. The platform could be rotated in two orthogonal great circles describing the coordinate system (θ, ϕ) in Fig. 1 *a*. The stimulus was supplied by the same apparatus as described for the retinular cell experiments and the stimulus beam passed through the center of the coordinate system occupied by the fly's head. Since the beam diameter was greater than the eye, it was possible to align the receptive field axis with the beam by rotating the preparation rather than the stimulus. This was made possible by the exceptional stability of the recordings.

Selective Motion Detection Experiments

Spike potentials from units sensitive to select directions of motion were recorded from the third optic ganglion and cervical connective of *Musca domestica* and *Phaenicia sericata* with stainless steel microelectrodes and have previously been described as Class II and Class IV units, respectively (16–18). The optomotor torque measurements were made with a torquemeter having high accuracy from DC to 100 Hz (19).

Since all of these responses were selectively sensitive to motion, a basic stimulus was used which consisted of alternate dark and light stripes moving at a constant velocity in a direction perpendicular to the stripes. The parameters defining such a stimulus are

(*a*) pattern size, shape, and direction of motion;

(*b*) spatial wavelength (λ_s);

(*c*) intensity of light stripes (I_1) and dark stripes (I_2) (contrast ratio $= \frac{I_1 - I_2}{(I_1 + I_2)}$); and

(*d*) angular velocity (V) (contrast frequency $= V\lambda_s$).

To provide such a stimulus pattern of monochromatic light over both the visible and ultraviolet regions, the apparatus shown in Fig. 1 *b* was used. The light from a 300 w xenon arc lamp was first collimated and filtered of much of its infrared component. The spectral composition of the light was determined by one of 20 interference filters (F_2) and the intensity was controlled by inserting neutral density filters (N). The stimulus beam was directed at a quartz diffuser (D) placed close enough to the preparation so that the illuminated area represented 30° of arc to the fly. Moving stripes were generated by placing between the diffuser and the preparation an interchangeable drum of alternate bars and slits. The drum velocity and direction of motion were controlled electrically. Uniform light to change the contrast ratio or for selective

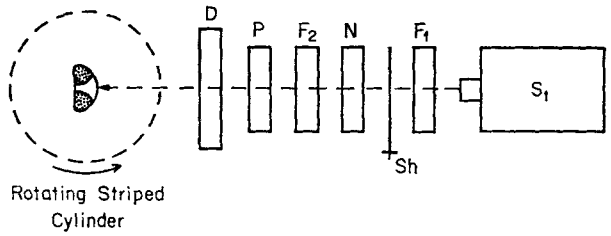

FIGURE 1 *b*. Schematic diagram of the second stimulus system. See legend for Fig. 1 *a* for definition of abbreviations. System B was used in motion detection and optomotor experiments.

adaptation was projected upon the eye by a second beam which bypassed the moving cylinder.

Data Analysis

The data from all experiments were analyzed with the biological data processing system developed at the California Institute of Technology (20). Data from experiments on retinular cells and intermediate chiasma units were recorded on analogue tape and subsequently reproduced for off-line analysis. The selective motion detection units and flight torques, however, were analyzed on-line and the results of the analysis were used to control the experiments. Typically, such an experiment was performed by presenting a sequential pair of alternate stimuli identical except for their spectral wavelengths and intensities. The computer continually updated the average time history of the pair of responses and graphically displayed them together with the total number of spikes generated during a given period of the stimuli in the case of neuronal units and the average torques in the case of optomotor responses. In starting an experiment an initial guess was made of the relative intensities of the two patterns required for equal responses. After a series of four or five sets of stimuli, the response imbalance as indicated by the two computations was used to determine the intensity readjustment. This process was repeated until a balance was obtained. The relative spectral sensitivity for these two wavelengths was then calculated from the balance intensities.

RESULTS

Spectral Sensitivity of Retinular Cells

It is important in studies of sensory neural processing to identify the modes by which the observed signals carry information. In the case of slow potentials, latency and magnitude of the response represent simple codes; however, information could likewise be more complexly coded in the time-course of the response. In a single response it is possible to encode both the wavelength and intensity of the stimulus, for example, by coding wavelength with latency and, independently, intensity with magnitude. Such a signal would have bivariant properties (two degrees of freedom). A more realistic example occurs when intensity and wavelength are not coded independently. Another problem

associated with neural coding is a consequence of the single unit analysis approach, for knowledge of a single unit's response is not necessarily sufficient to decipher the message. In other words, the code may be distributed among the responses of more than a single unit.

The first step in the study of spectral and polarization properties of retinular cells was to determine the number of degrees of freedom in the response of a single retinular cell. Three attributes of the stimulus (intensity, wavelength, and polarization angle) were considered. For every retinular cell recorded it was always possible to make its response to different polarizations and wavelengths temporally congruous merely by adjusting the intensity. An example of the temporal congruity of retinular cell responses is shown in Fig. 2 in the

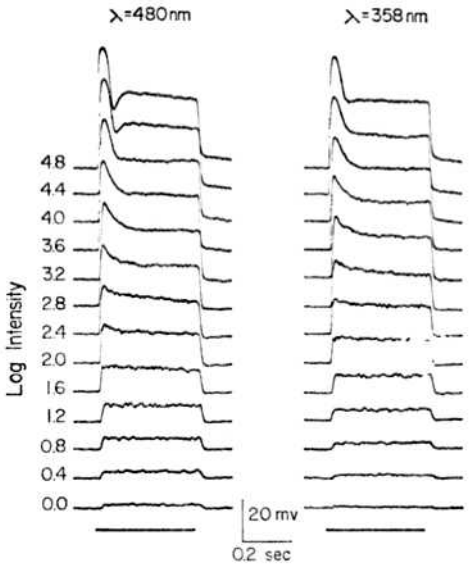

FIGURE 2. Retinular cell responses to a sequence of increasing intensity flashes having wavelengths of 480 and 358 nm. The line segment below each column of responses represents the stimulus presentation. The two families of responses can be made nearly congruous by shifting the 480 nm family up one intensity level.

case of two stimuli differing in wavelength. The responses were obtained by first presenting at the lowest wedge setting (log I = 0) a monochromatic stimulus of 480 nm and then of 358 nm followed subsequently by such pairs at successively higher wedge settings. At any given intensity level, the 480 nm response is not identical to the 358 nm response due to the difference in the source efficiency and the fly's sensitivity at the two wavelengths; however, it can be identically matched (within error of the response) to one at 358 nm by appropriately adjusting the intensity at this wavelength. The intensity adjustment necessary to produce temporal congruity at these two wave-

lengths is the same regardless of the intensity level of the 480 nm stimulus. This can be seen from the data in Fig. 2, for temporal congruity can nearly be obtained by shifting the 480 nm column up one intensity level. Better congruity is actually achieved in this case by making the 480 nm stimulation 0.5 log units less intense. Such temporal congruity as this was possible for all combinations of wavelength and polarization angle. Since the response of every retinular cell regardless of the attributes of the stimulus eliciting it can be characterized by a single intensity value, the response is univariant (i.e., one degree of freedom).

Although information about stimulus intensity, wavelength, and polarization was not distinguishable from a single retinular cell response, it may be distributed over a population of retinular cells. To assess this possibility a statistical strategy was employed whereby the spectral and polarization properties of many cells were sequentially sampled. Spectral and polarization properties of retinular cells were characterized by their spectral and polarization sensitivity which is defined as the inverse of the light intensity, measured in photons, required to elicit a criterion response at each sample wavelength or polarization angle. Univariant responses are particularly well suited for sensitivity measurements, for all responses regardless of the stimulus attributes can be characterized by a single parameter, light intensity. Experimentally, spectral sensitivity measurements were determined from the retinular cell responses elicited by a series of monochromatic flashes of sequentially greater intensity, called a log I series, and a series of monochromatic flashes of different wavelengths at a fixed wedge position. Using such experimental data, the spectral calibration of the source, and univariance, it was a simple matter to calculate spectral sensitivities. A total of 27 retinular cells were studied in this fashion, and all were found to possess the same spectral sensitivity. Hypothesis testing was not necessary to verify this, for all cells responded quite similarly. The cells were predominately recorded from the medioventral quadrant of the retina. The average spectral sensitivity of the retinular cells is shown in Fig. 5. The standard deviation of the normalized spectral sensitivity measurements at all of the 12 sample wavelengths was less than five percentage points.

The simplest explanation for the two peaks in the spectral sensitivity is the existence in each retinular cell of a single type of photopigment having two absorption maxima. Nevertheless, there are several alternative explanations involving two photopigments (i.e., a UV and a green type). For example, some retinular cells in a single ommatidium might possess UV photopigments while others possess green photopigments, and by physiological or nonphysiological coupling of such cells, a double-peaked spectral sensitivity would result. Such a possibility would not necessarily violate the observed univariance if both types of cells had the same temporal response characteristics.

To test this possibility spectral adaptation experiments were performed by recording the spectral responses of retinular cells in the dark- and light-adapted states. The adapting light had a wavelength of 501 nm and an intensity sufficient to produce a steady 15–20 mv depolarization from the dark resting level. The experiments indicated that the steady adapting light reduced the sensitivity of the cells to all wavelength flashes equally, which does not support the hypothesis of two types of retinular cells. Under such a hypothesis, the green adapting light would be expected to reduce the sensitivity of green-type retinular cells more than UV-type retinular cells.

Some of the spectral sensitivity experiments were performed with micropipettes filled with Procion yellow dye so that the cell from which the records were obtained could be histologically identified. Of more than 20 retinular cells stained, 12 cells were stained completely enough to identify them positively as being one of retinular cells 1 to 6. Examples of two of the best-stained retinular cells are shown in the montages of Fig. 3. Due to the length of the cell and projection pattern of its axon, a complete stain was never contained in a single 15 μ section. The axon endings in the first optic ganglion positively identify these cells as belonging to retinular cells 1 to 6 and the diameter, length, and the swelling near the pseudocones at the level of the cell nucleus provide additional support for this identification. A stained cell was never observed which could be correlated with the morphology of retinular cells 7 and 8. Several different retinular cell axon projection patterns were observed in the first optic ganglion, which suggests that several and probably all of the retinular cells 1 to 6 were studied. Since all retinular cells including those stained had the same spectral sensitivity, it follows that retinular cells 1 to 6 have the spectral sensitivity shown in Fig. 5. Dye leakage into adjacent retinular cells, which is suggestive of a nonphysiological coupling resulting from microelectrode damage, was never observed.

Spectral Sensitivity of Chiasma Units

Spike potentials from two classes of units can be recorded from the intermediate chiasma of flies (21). The spikes were centripetal and reflect the integrating properties of the first optic ganglion. The two classes of units were referred to as sustaining and on-off units, and their spatial and temporal integrating properties are fully described elsewhere.[1] In brief, the sustaining units have a receptive field composed of three roughly circular regions arranged adjacently along a line parallel to the mediolateral axis of the eye. The center region is excitatory and supports a sustained discharge while both adjacent regions are inhibitory and support an "off" discharge. The on-off

[1]Arnett, D. W. 1972. Spatial and temporal integration properties of units in the first optic ganglion of diptera. *J. Neurophysiol.* In press.

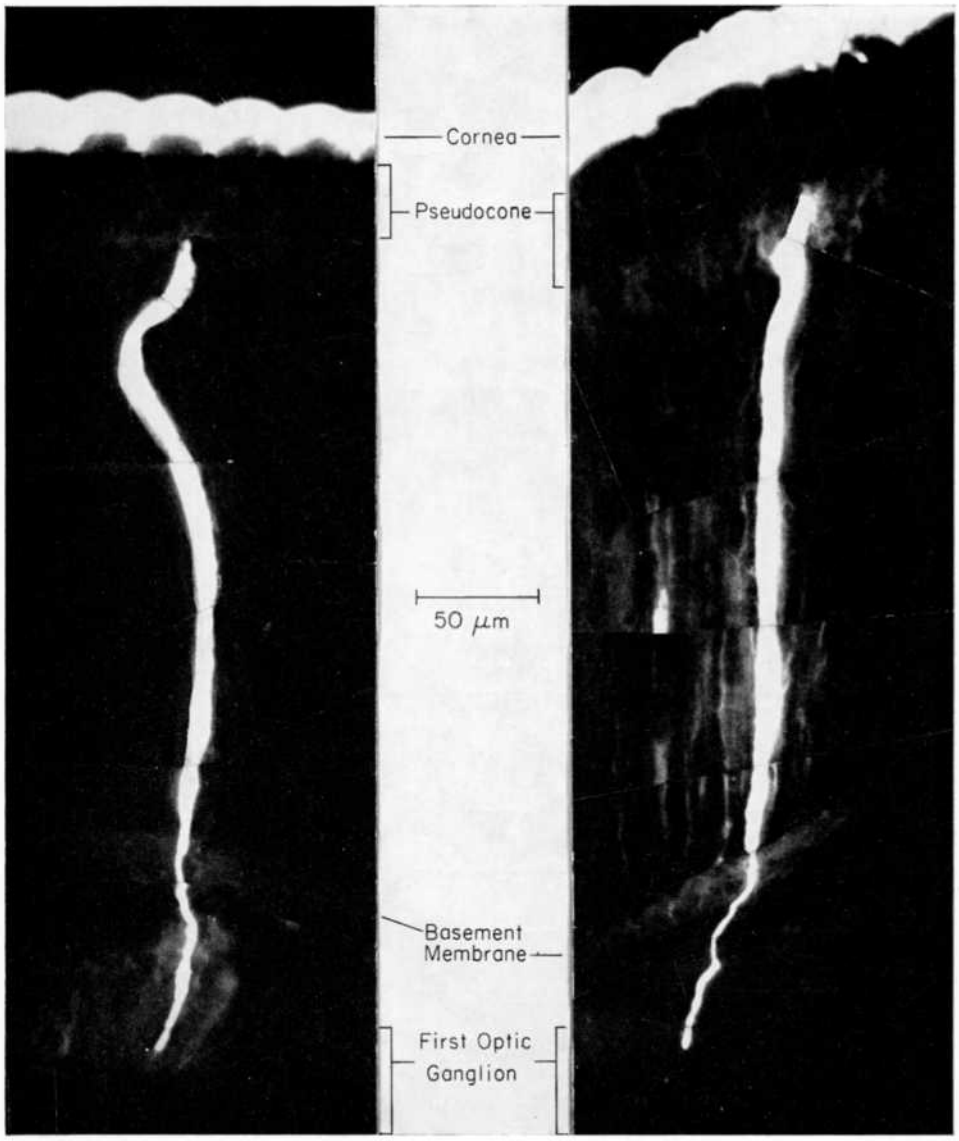

FIGURE 3. Photomontages of two Procion yellow–stained retinular cells. Both cells were stained by passing a cathodal current of 5 na for 2 min. Distortions in the retina occurred during histological processing. The left cell was embedded in Maraglas while the right cell was embedded in paraffin and sections were made at 15 μ.

units have elliptical receptive fields, anywhere within which an "on-off" discharge can be elicited.

Typical average discharge responses for both types of unit are shown in Fig. 4. The data were obtained from experiments similar to the temporal

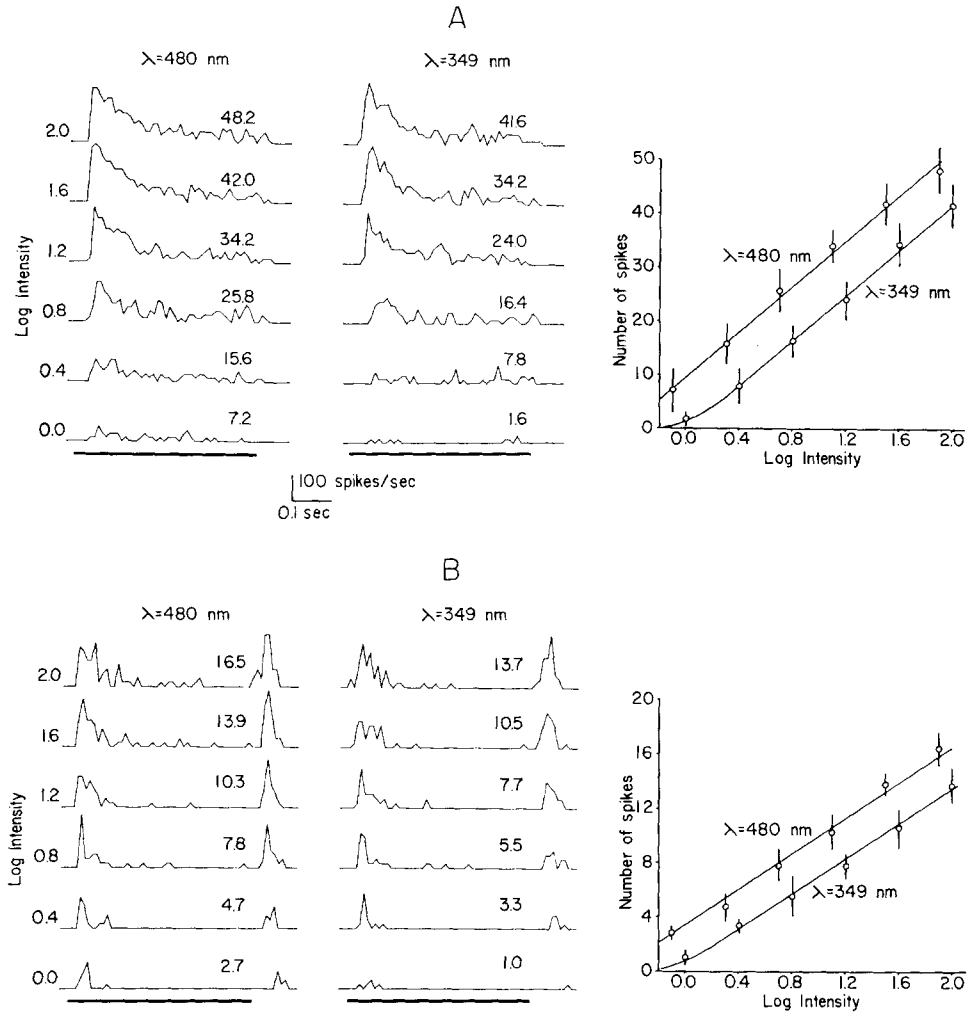

FIGURE 4. Average responses and response–log intensity relations for typical sustaining (A) and on-off (B) units of *Phaenicia sericata*. Five trials contributed to each average and the number above each response refers to the average number of spikes per stimulus. These numbers and ±1 SD are plotted on the right-hand axes. The line segment below each family represents the stimulus presentation. In both (A) and (B) the 480 nm response–log intensity curves were shifted to the left by 0.1 log unit for added clarity. Time interval of poststimulus time (PST) histogram (bin width) is 10 msec.

congruity experiments on retinular cells (Fig. 1), except that the intensity range was only one-half and five repetitions were made to form the average. Averaging was necessary due to the relatively small amount of data contained in each discharge response as compared to that in a slow potential response. Considerable averaging would be necessary to approach the signal-to-noise ratio of slow potentials; however, within the limits of the noise in the average

discharge responses shown in Fig. 4, temporal congruity can be demonstrated for any combination of wavelength and polarization for both types of unit. As with retinular cells, this implies response univariance which is manifested in the parallel nature of the response (average number of spikes per stimulus)–log intensity curves of Fig. 4. In this particular case temporal congruity can be approximately achieved by making the 349 nm stimulus 0.4 log unit more intense than the 480 nm stimulus. Note that the log-linear range of these responses has not been exceeded by the maximum intensity stimulus.

Two methods were used to measure the spectral sensitivity of sustaining and on-off units. The first method was identical to that used for retinular cells except that averaged discharge responses were used instead of slow potentials; however, this method was subject to greater error due to the fluctuations in the averaged responses. The second method which was primarily used employed the fact that both units were quiescent in the dark, thereby making a single spike a well-defined criterion response. The stimulus intensity necessary to elicit a threshold response (one spike) was determined for each wavelength, and, to avoid error introduced by slow changes in the absolute sensitivity of the eye, each measurement was made relative to a control wavelength (480 nm).

As in the case of retinular cells, all units of both types possessed the same spectral sensitivity. Although the threshold method yielded measurements with less variance, there was good agreement between the measurements by the two methods. The average spectral sensitivities from 26 sustaining units and 38 on-off units are shown in Fig. 5. As can be seen, the spectral sensitivities of retinular cells 1 to 6, sustaining units, and on-off units are in close agreement.

Since on-off units are also quiescent in the presence of steady illumination, spectral sensitivity measurements were made on these units by the threshold method in the presence of an adapting light with a wavelength of 501 nm. The adapting light was found to lower the threshold at each wavelength by the same amount (up to 2 log units) depending upon the intensity of the adapting light. As in the case of retinular cells, no change in the form of the spectral sensitivity was observed under light adaptation conditions.

Spectral Sensitivity of Class II Units

Selective motion detection units (Classes II and IV) have well-defined response variations with the form and motion properties of visual stimuli (16, 17), and a detailed study was made to determine the effects of spectral wavelength on the variations. The study was made by suddenly applying a 30° in diameter circular pattern of stripes moving at a constant velocity. Important parameters of this pattern which specify its form and motion properties are pattern spatial wavelength, velocity, contrast ratio, and background intensity.

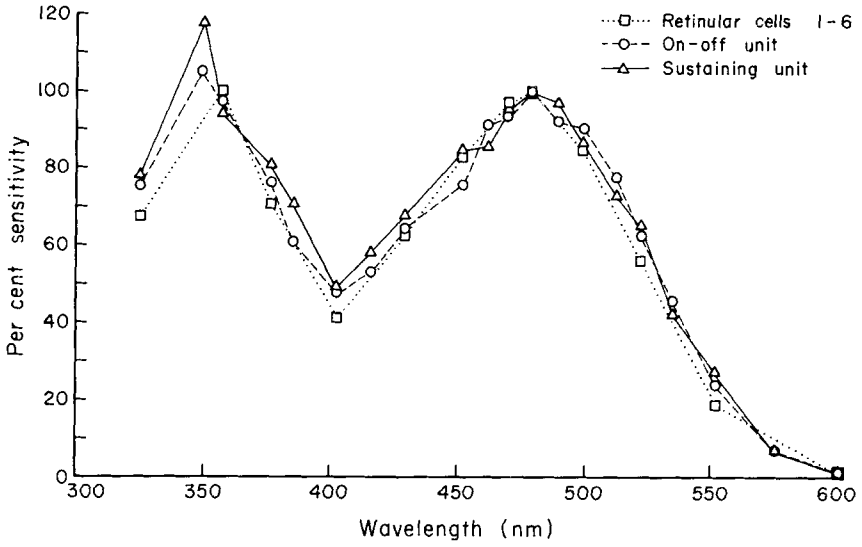

FIGURE 5. The average spectral sensitivity of retinular cells 1 to 6, sustaining units, and on-off units. The ordinate represents the normalized reciprocal of the number of photons necessary to elicit a criterion response. Only half as many sample wavelengths were used in the retinular cell study. Retinular cell data were obtained from white-eyed *Calliphora erythrocephala*, and sustaining and on-off unit data were obtained from wild-type *Phaenicia sericata* and *Calliphora erythrocephala*.

Comparisons of the complete transient responses were made as these parameters and spectral wavelength were varied.

As described in Materials and Methods, two patterns with identical form and motion properties but having different spectral wavelengths were alternately presented, and their relative intensities were adjusted in an attempt to obtain identical averaged responses. Typical results from such experiments are shown in Fig. 6. These averaged responses illustrate the degree of identity obtained when the relative intensities of the two patterns were correctly balanced. In Fig. 6 *c* the average responses for both forward and reverse motion are shown. It was always found that the same intensity ratio produced a balance for forward and reverse motion.

Spectral sensitivity measurements require a high degree of accuracy, and the balancing technique provides that accuracy. The accuracy depends upon the form of the response *versus* intensity relationship shown in Fig. 7 *a*. The slope of this curve determines the sensitivity of balance. Due to the extensive spatial summation of selective motion detection units, the response of the unit is usually saturated by stimulus intensities more than 1.5 log units above threshold, which implies a steep slope and consequently high balance sensitivity. The sensitivity of balance is shown for a typical unit in Fig. 7 *b* and was measured by perturbing the balance conditions and observing the re-

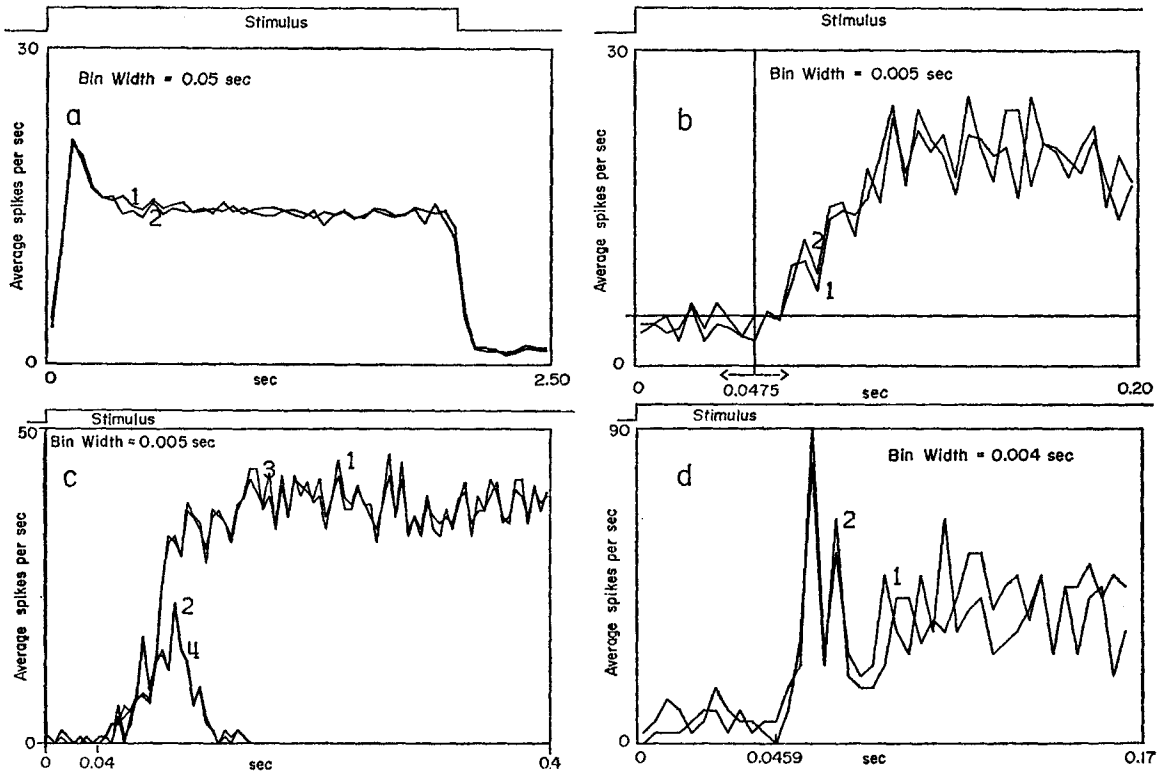

FIGURE 6. Typical balanced responses of Class II units of *Phaenicia sericata*. The averages were accumulated from the responses elicited by repeated presentation of a pair of constant velocity striped patterns differing only in their light wavelength and intensity. In all cases the spatial wavelength was 20°, and the contrast frequency was 2.8 Hz. (*a*) Curve *1*, λ = 526 nm; curve *2*, λ = 407 nm. Contrast ratio = 0.5; intensity $\approx$ three times threshold. Number of averages, N, = 95; total spikes in 2 sec for *1* = 2852, for *2* = 2841. (*b*) Same as Fig. 6 *a* but finer resolution. (*c*) Curves *1* and *2* are forward and reverse responses for λ = 554 nm. Curves *3* and *4*, λ = 454 nm; contrast ratio = 1.0; intensity $\approx$ five times threshold, N = 300; total spikes in 3 sec for *1* = 35,126; for *3* = 35,151. (*d*) Curve *1*, λ = 512 nm; curve *2*, λ = 350 nm. Contrast ratio = 1; intensity $\approx$ five times threshold; N = 36. Total spikes in 2 sec for *1* = 2737, for *2* = 2721.

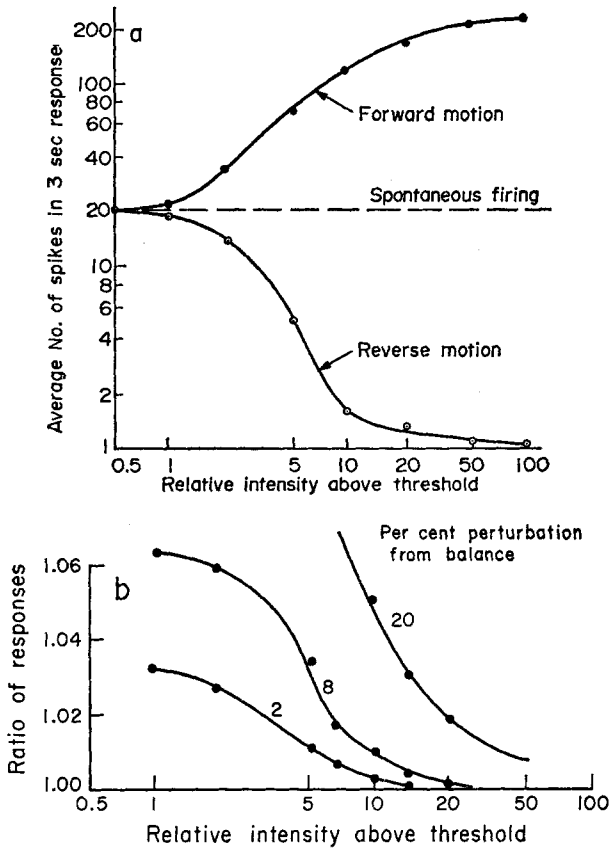

FIGURE 7. Typical response variation of the Class II unit in *Phaenicia sericata* as a function of pattern intensity. Spatial wavelength = 20°; contrast ratio = 1; white light illumination was used. (*a*) Response as a function of pattern intensity. (*b*) Response imbalance as a function of intensity imbalance.

sponse imbalance for various levels of intensity. The balance sensitivity is greatest near threshold and is steadily reduced as the response approaches saturation.

The spatial wavelength was found to be the most important pattern parameter with regard to its effect on the spectral sensitivity. Fig. 8 summarizes the variations of the Class II forward responses with spatial wavelength when white light patterns are used. These data were obtained by alternately presenting two to four stimuli of different spatial wavelength. Each pattern was presented for 3 sec with an intervening 10 sec between patterns, and the relative intensities of the patterns were adjusted until the total spikes generated in each 3 sec period balanced to within 1%. A sufficient number of repetitions were performed so that the total spikes for each case was at least 300.

Two regions of distinctly different response properties are apparent. For

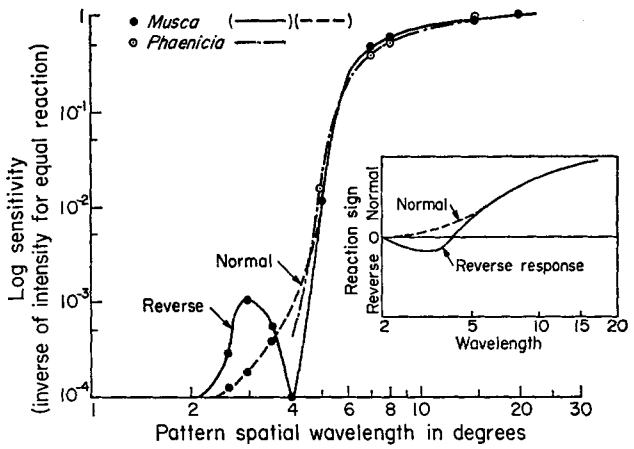

FIGURE 8. Response variation as a function of pattern spatial wavelength. Contrast ratio = 1; contrast frequency = 2.8 Hz.; white light illumination was used. The *inset* illustrates the range of responses obtained at short spatial wavelengths. The continuous line illustrates the response variation when a reverse response could be elicited in *Musca* and the broken line illustrates the variation when only a normal response could be obtained.

λ_s greater than about 5°, normal forward motion responses were obtained with consistent response variations as a function of λ_s. No differences could be found for any of the flies studied or between any of the Class II and Class IV neuronal units. For spatial wavelengths between 2° and 4° and for certain *Musca* preparations, the unit was excited only when the direction of pattern motion was reversed from that for long spatial wavelength (stroboscopic effect). Such reverse reactions could not be obtained for *Calliphora* or *Phaenicia* and were only induced in about one-half of the *Musca* preparations. The above two regions of λ_s provided, in the case of the *Musca* preparations, different results as the wavelength of monochromatic light was varied, and they will be discussed separately.

Patterns with Long Spatial Wavelengths

Experiments like those illustrated in Fig. 6 were run using spatial wavelengths of 7.5°, 14.5°, 20°, and 45° for all three species of flies (both wild-type and white-eyed). In all cases it was found that for a given pair of light wavelengths only a single intensity ratio was necessary to produce a balance. The spectral sensitivities that resulted from this study are illustrated by the curve of Fig. 9 for λ_s = 20°. This curve was found to apply for all values of intensities from threshold to saturation and for contrast ratios from 0.10 to 1. Selective adaptation at monochromatic wavelengths of 465 and 510 nm also showed no effect on the spectral sensitivity.

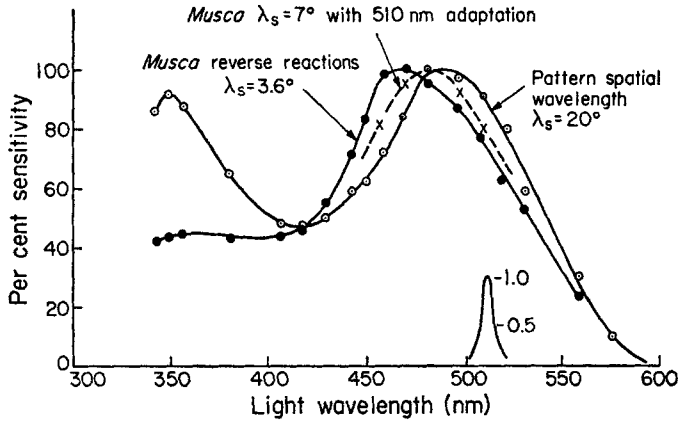

FIGURE 9. Average spectral sensitivity curves for the Class II unit. Filled circles represent the spectral sensitivity obtained when using short spatial wavelength patterns. Open circles represent the spectral sensitivity at long spatial wavelengths. Crosses represent the spectral sensitivity obtained using a pattern with a spatial wavelength of 7° in the presence of a steady monochromatic adapting light of 510 nm. The small narrow peaked curve represents the approximate transmission characteristics of the visible region interference filters.

Patterns with Short Spatial Wavelengths

Pattern intensities of more than 100 times the threshold level of the long wavelength patterns were required to elicit the reverse reactions from *Musca*. These studies were made using values of λ_s of 2.6° and 3.6°. Although the responses were much more variable, reasonably good balances could be obtained for each pair of light wavelengths used in a single intensity ratio was again obtained for each combination. However, the resulting spectral sensitivity was different as shown in Fig. 9 (λ_s = 3.6°). Spectral and white light adaption merely decreased the sensitivity at all light wavelengths equally.

The results of Fig. 9 indicate that only retinular cells 1 to 6 are contributing to the responses of the long spatial wavelength patterns and that only retinular cells 7 and 8 are contributing to the reverse reaction responses of *Musca* for the very short spatial wavelength patterns. It was considered possible to find a contribution from both systems in *Musca* if a spatial wavelength at the lower end of the longer wavelength range was used, and a strong green background adapting light was employed to reduce the sensitivity of the (1–6) subsystem. The only preparations that gave a spectral sensitivity curve different than that for the (1–6) subsystem were those for which a reverse reaction could be obtained with the very short spatial wavelengths. A typical result is shown by the (λ_s = 7°) curve of Fig. 9. Before applying the adapting light, the mono-

chromatic peak spectral sensitivity was located at 480 nm, and the amount of the shift toward 465 nm was a function of the adapting light intensity.

Spectral Sensitivity Studies of Class IV Units and Optomotor Responses

The experiments described above with the two ranges of pattern spatial wavelengths were also made using the Class IV responses. No differences in the spectral sensitivities could be found from those for Class II unit responses. Similar tests were made on the optomotor yaw torque reaction of *Musca*. For this case the moving pattern (using one light wavelength) was run steadily 10 sec in one direction, then suddenly reversed for 10 sec. After a 10 sec interval the cycle was repeated with a different light wavelength and successive pairs of these tests were multiplexed. The relative intensities were adjusted so that the average peak-to-peak responses were equal. Such tests performed on *Musca* produced the same three curves of Fig. 9.

Polarization Sensitivity

All retinular cells studied exhibited sensitivity to the plane of polarization. The effect was noticeable but not great and seldom represented an effective intensity change of greater than 0.3 log unit. The preferred planes of polarization varied from one retinular cell to the next, but they seemed to fall into three groups. However, a quantative correlation between preferred planes of polarization and retinal geometry was not attempted.

In contrast to the situation for retinular cells, no indication of polarization sensitivity was observed for any of the sustaining or on-off units. Similarly the Class II units, the Class IV units, and the optomotor responses showed no indication of polarization sensitivities when tested with the long spatial wavelength patterns. However, the reverse reactions of *Musca* to very short spatial wavelengths patterns can be shown to exhibit variations with the orientation of polarized light stimuli. This was found for both the Class II units and the optomotor reactions, and the effect varied greatly with the size and orientation of the pattern since the orientation of the microvilli of retinular cells 7 and 8 varies considerably over the eye. Detectable effects were found for a rectangular pattern 5° high by 10° in the direction of motion. Patterns of spatial wavelength of 2.6° and 3.6° that elicited reverse reactions showed response variations with polarization angle for both broad-band and monochromatic light with wavelengths between 420 and 550 nm. However, no such variation could be detected in the ultraviolet region. The response fluctuation followed an approximate sine law with maxima and minima 90° apart. The fluctuation was detectable only near threshold intensity and was quite small, corresponding to an effective intensity change of only about 0.2 log unit.

DISCUSSION

Visual Subsystem Hypothesis

The correlation of these functional studies with anatomical data shows that the retinas of flies can be subdivided into two parts. One part consists of retinular cells 1 to 6 of each ommatidium whose axons terminate in the first optic ganglion, and the other consists of retinular cells 7 and 8 (superior and inferior central cells) of each ommatidium whose axons appear from light and electron microscopy (22, 23) to bypass the first optic ganglion and to end in the second optic ganglion. This dichotomy can be extended to include other levels of the visual system as well, in which case the visual nervous system would be comprised of a (1–6) subsystem served by retinular cells 1 to 6 and a (7, 8) subsystem served by retinular cells 7 and 8. The two systems are not isolated, for at each point studied functional contributions could be traced back to either retinular cells 1 to 6, or to cells 7 and 8, or to both.

Functional studies at various levels of the visual system have provided support for other aspects of this hypothesis advanced from purely anatomical considerations. The microspectrophotometric measurements (6, 7) on individual rhabdomeres revealing that the two types of retinular cells also differ with respect to their spectral absorption characteristics were confirmed indirectly by our study, for the Class II units, Class IV units, and optomotor responses were shown to be under the influence of both the (1–6) and the (7, 8) subsystems. Eckert (1) also found this to be true for optomotor responses.

The rationale behind the method used for accentuating the individual contributions to motion detection from the (1–6) subsystem or the (7, 8) subsystem is as follows. The rhabdomeres of retinular cells 7 and 8 are smaller than those of retinular cells 1 to 6, which implies that they are characterized by a smaller receptive field and hence finer resolution. However, the (7, 8) subsystem has lower absolute sensitivity, for the rhabdomeres of retinular cells 7 and 8 would not be expected to capture as many photons as those of retinular cells 1 to 6 due to their smaller cross-section and shorter length (24). Furthermore, the (1–6) subsystem has a sixfold convergence in the first optic ganglion. In total, it is estimated that the (1–6) subsystem is between 10 and 100 times more sensitive than the (7, 8) subsystem. Since the receptive field sizes of retinular cells 1 to 6 and cells 7 and 8 differ, their attenuation characteristics (attenuation of response to a moving striped pattern as a function of spatial wavelength) also differ as illustrated in Fig. 10 A. Taking into consideration the sensitivity difference, the relative contributions of the two subsystems as a function of spatial wavelength would appear as in Fig. 10 B. Clearly, the contributions from the (1–6) subsystem should dominate for larger spatial wavelengths, and those of the (7, 8) subsystem should dominate for

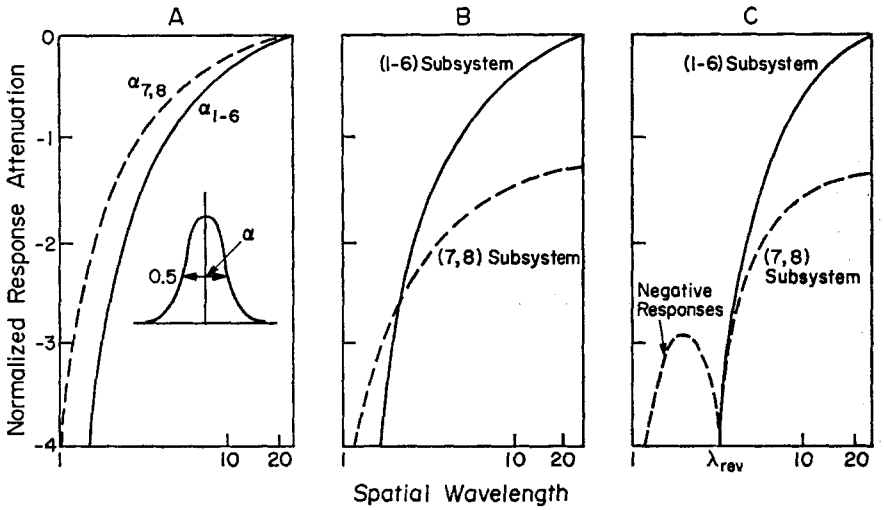

FIGURE 10. Participation of the (1–6) and (7, 8) subsystems in the motion detection process. (A) Probable response attenuation curves for retinular cells 1 to 6 and cells 7 and 8 as a function of λ_s. The half-sensitivity angle (α) is smaller for retinular cells 7 and 8 than for cells 1 to 6. (B) Expected performance of the two subsystems assuming algebraic summation of retinular cell signals. (C) Expected contribution to motion detection by each subsystem.

very small spatial wavelengths. Therefore, neural units whose responses result from essentially algebraic summation of the contributions from both subsystems should have attenuation characteristics like those in Fig. 10 B. However, the response of Class II units, Class IV units, and the optomotor torque has an added complexity resulting from the temporal correlation mechanism inherent in the detection of motion. Based on the correlation model of motion detection (25), the predicted attenuation of the contribution to motion detection by both subsystems is as shown in Fig. 10 C. For spatial wavelengths shorter than λ_{rev} reverse or negative responses are obtained (i.e., the responses indicate that the direction of perceived motion is opposite to the true direction of motion, or the stroboscopic effect). The value of λ_{rev} for motion detection reversal is determined by the effective angle between the optical axes of adjacent retinular cells contributing to the reaction. Since these angles are the same for the (1–6) and (7, 8) subsystems (26), contributions from either subsystem should have the same λ_{rev}. Since contributions from the (1–6) subsystem are more rapidly attenuated with shorter spatial wavelengths than are those of the (7, 8) subsystem, the only detectable contribution to the reverse motion reaction at small spatial wavelengths is from the (7, 8) subsystem. The fact that spectral and polarization measurements made for spatial wavelengths greater than λ_{rev} revealed properties that can be attributed to only the (1–6) subsystem while those for spatial wavelengths

less than λ_{rev} can only be attributed to the (7, 8) subsystem supports the above analysis.

Direct evidence of retinal dichotomy was frustrated by the failure to record intracellular potentials from retinular cells 7 and 8. This failure was presumably due to their much smaller size in comparison to retinular cells 1 to 6. Since all retinular cells 1 to 6 are the same size and since all retinular cells studied, including those positively identified as a retinular cell 1 to 6, possessed the same spectral sensitivity, it is reasonable to believe that all retinular cells 1 to 6 possess the same spectral sensitivity in accordance with the spectral absorption results.

Substantiation that the first optic ganglion belongs completely to the (1–6) subsystem was obtained functionally since the sustaining and on-off units which reflect integrative properties of the first optic ganglion possessed the same spectral sensitivity as retinular cells 1 to 6. This also tends to reinforce the conclusion that all retinular cells 1 to 6 have the same spectral sensitivity since no evidence of color coding mechanisms, as are present in some fish (27, 28), or of different spectral sensitivities was obtained from on-off and sustaining units.

Interaction between the two subsystems was first encountered in directional selective motion detection units of the third optic ganglion, although interaction probably occurs in the second optic ganglion where the axons of retinular cells 7 and 8 are known to terminate. Interaction between the two subsystems at the Class II and IV unit and optomotor levels seems to be limited to simple summation. In all cases the influence of the (1–6) subsystem greatly dominated, and it was only under very special conditions that the influence of the (7, 8) subsystem could be observed.

Spectral Sensitivity

Our results indicate that all retinular cells 1 to 6 have the same spectral sensitivity which differs from that of retinular cells 7 and 8. There is some disagreement among the findings of other investigations with respect to the locations of the maximum spectral sensitivity. Our results indicate that retinular cells 1 to 6 have a spectral sensitivity characterized by two maxima of approximately equal sensitivity located at 480 and 350 nm, while retinular cells 7 and 8 have a spectral sensitivity with a single maximum at 465 nm.

This is in very good agreement with the results reported by Eckert (1), who also found two maxima for the (1–6) subsystem located at 485 nm and approximately 360 nm and a single maximum at 465 nm for the (7, 8) subsystem. Spectral sensitivity measurements from single retinular cells of flies were first made by Burkhardt (4), who reported three populations having different spectral sensitivities. The most common retinular cell possessed a spectral sensitivity maximum at 350 nm and at 490 nm and, in light of our results,

most probably corresponded to retinular cells 1 to 6. Retinular cells belonging to the other two populations were recorded much less frequently and one type had sensitivity maxima at 350 and 470 nm while the other type had maxima at 350 and 521 nm. Although it seems unlikely, it is possible that the retinular cell population having a 470 nm maximum corresponded with retinular cells 7 and 8. The greatest disagreement is introduced by the microspectrophotometric measurements. Langer and Thorell (6, 7) found rhabdomeres of retinular cells 1 to 6 to have a major absorption peak at 515 nm and a minor peak near 385 nm while the rhabdomere complex of retinular cells 7 and 8 had a major absorption peak at 470 nm and a minor peak near 400 nm. In comparison, the spectral sensitivity measurements based on neurophysiological studies, all of which are in very close agreement, indicate that retinular cells 1 to 6 have two maxima of approximately equal sensitivity located near 350 and 485 nm while retinular cells 7 and 8 have a single maximum located near 465 nm. It is not surprising to find a quantitative difference between the spectral absorption and functional sensitivity measurements since the various methods are quite different and subject to several sources of error.

Clearly, retinular cells 1 to 6 and cells 7 and 8 comprised spectrally different populations, which satisfies a necessary condition for wavelength discrimination. The difference between the spectral sensitivities of the two populations, with the exception of the UV region, however, is very small. For this reason an ability to discriminate different wavelengths seems remote unless considerable neural processing takes place. Such processing was not observed at the points in the nervous system which we studied. However, this is not surprising, for the first optic ganglion belongs completely to the (1–6) subsystem and motion detection is likely independent of wavelength discrimination.

UV Sensitivity

Many insect retinas contain retinular cells maximally sensitive to ultraviolet stimulation (350 nm) which suggests that they contain a UV photopigment. In flies, this is not the case, for although retinular cells 1 to 6 have a sensitivity peak at 350 nm, they have one in the green region (480 nm) of the spectrum as well. Spectral sensitivities with two maxima have also been obtained from honeybees (29), locusts (30), and dragonflies (31, 32). In both honeybees and dragonflies, however, retinular cells with a single spectral sensitivity maximum coinciding with the locations of one or the other of the two maxima were also observed. Furthermore, the relative sensitivities of the two maxima for the double maximum retinular cells were not fixed. It was suggested that the spectral sensitivities with two maxima resulted from a nonphysiological coupling between adjacent retinular cells brought about by the micropipette impaling successive cells. The coupling may also have been physiological since Shaw (33) has shown adjacent retinular cells in the honeybee drone to

be coupled. In any case, the two maxima seem to be related to the activity of two populations of retinular cells containing two different photopigments. These explanations do not hold up in the case of the fly, for the relative magnitudes of the two maxima were rather constant, and retinular cells with a single maximum at 350 nm were never encountered. Most importantly, selective spectral adaptation experiments failed to show that the two maxima were a manifestation of coupling (physiological or nonphysiological) between hypothetical UV and green-type retinular cells. Furthermore, dye leakage from the impaled retinular cells into neighboring cells was never observed. Recently, the recordings of the double-peaked spectral sensitivities of dragonfly retinular cells have been attributed to an error introduced by off-axis stimulation (32). This explanation does not apply in our case for the visual axes of the cells were aligned to the stimulus beam before measurements were made.

Two possible explanations remain. Each cell could contain a complement of separate UV and green photopigments or each cell could contain a single type of photopigment having UV and green absorption maxima. Unless absorption of a photon by the UV and green photopigments led to temporally different responses, as they appear to do in the cockroach (34), the two possible explanations would be functionally indistinguishable, for spectral adaptation (as employed in our experiments) was thought to involve the cell membrane and not the photopigments as in bleaching adaptation. Since retinular cell responses are temporally congruous, a single photopigment with two absorption peaks was considered responsible. However, photopigments such as this have yet to be studied in vitro.

Polarization Sensitivity

Retinular cells 1 to 6 are selectively sensitive to the plane of polarized light. Sustaining and on-off units respond independently of the plane of polarization, yet both belong to the (1–6) subsystem. This verified the indication based on the neural anatomy of the first optic ganglion that the activity of retinular cells 1 to 6 is indiscriminately integrated in the first optic ganglion. An unsuccessful attempt was made to demonstrate this by selectively adapting sustaining and on-off units to various orientations of the plane of polarization. The failure was considered to be due to the small dichroic ratio which would not limit the adaptation to only those retinular cells having a preferred polarization plane that corresponded to the adapting plane. The loss of polarization discrimination from the (1–6) subsystem was further demonstrated by the higher order motion detection response which showed no polarization sensitivity when long spatial wavelengths stimuli were used. Polarization sensitivity was, however, detectable with very short spatial wavelength patterns in *Musca* for which the (7, 8) subsystem is presumed to provide the only con-

tribution. Variation of the response with maxima and minima 90° apart indicates that primarily one of the two retinular cells (7 or 8) is contributing to the reaction.

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